

INSIDE THE USCAP JOURNALS

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MODERN PATHOLOGY

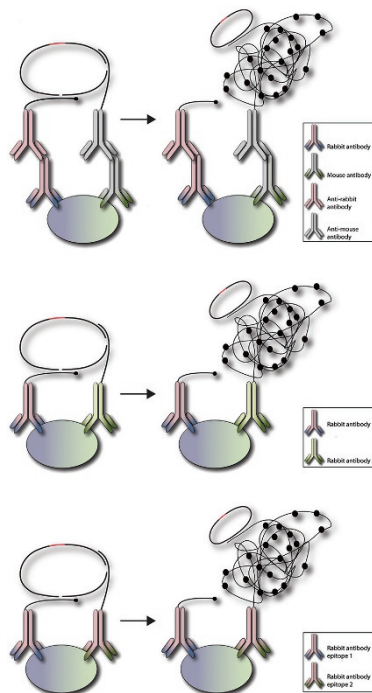
Specific protein detection

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Immunohistochemistry (IHC) is a critical *in situ* technique for protein detection in anatomical pathology and research. The technique hinges on antibodies designed to specifically bind to the protein of interest. One of its limitations has been that the available antibodies are not sufficiently sensitive or specific to optimize detection, especially that of proteins with low abundance.

Zieba *et al* sought to optimize both the antibodies and reagents involved in IHC. They conjugated DNA strands to the antibodies for optimal detection in order to avoid using antibodies prepared in a species different from that of the detection antibody, and tested several approaches to constructing reagents for IHC. By adding multiple antibodies to the assay, along with conjugated DNA strands to other versions of the assay, they were able to compare and optimize the assay.

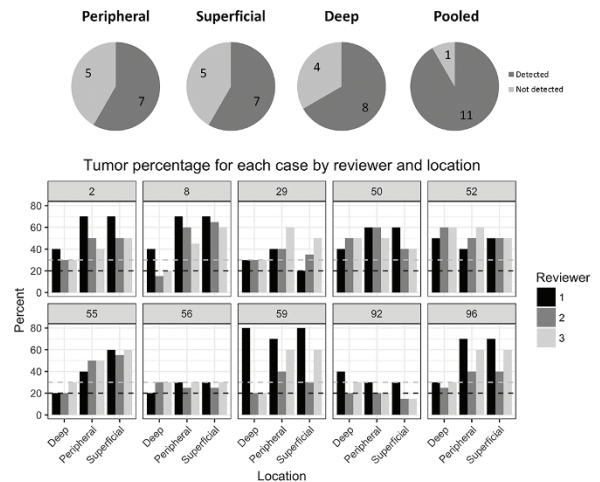
The group improved rabbit-derived antibodies by conjugating DNA strands to facilitate a proximity ligation assay with improved performance. These new assays provide improved performance, sensitivity, and specificity compared with IHC. With detailed protocols for direct labeling of antibodies, they provide a cost-effective method for pursuing pathological accuracy.



Sample pooling overcomes heterogeneity

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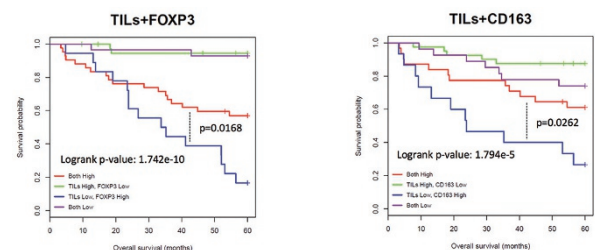
Intratumoral genomic heterogeneity, while known to influence cancer progression and therapy resistance, does not have a confirmed role in genomic testing and patient management. Therapy selection and clinical trial eligibility have begun being



managed with the use of genomic biomarkers, and an increasing emphasis in colorectal cancer has established that *KRAS* and *NRAS* mutations need to be documented for ideal management. Nelson *et al* performed Clinical Laboratory Improvement Amendments–compliant next-generation sequencing of peripheral, superficial, deep, and pooled samples from 99 cases of colorectal cancer. Twelve cases failed quality control and 25 were negative for mutations; the remaining 62 cases had a mutation in at least one sample. Mutations in *KRAS* were most prevalent (found in 33% of cases), followed by *BRAF* and *PIK3CA*. The group defined a discordant case as one in which a specific mutation was detected in at least one site (peripheral, superficial, or deep) but not in at least one different site. Ten of the 12 discordant mutations were in *PIK3CA*; the remaining 2 were in *KRAS*. The authors concluded that this pooling strategy could overcome issues of poor tumor-mass representation.

Immunological landscape of breast cancer

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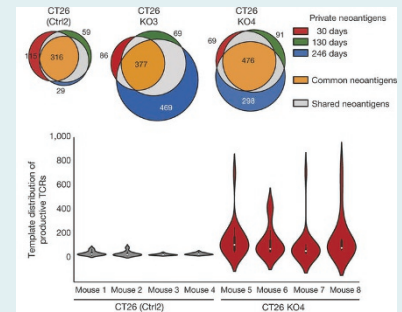


Adams *et al* investigated the interrelationship of tumor-infiltrating lymphocytes, immune response regulators,

DNA mismatch-repair deficiency impairs tumor growth

Germano *et al* investigated the functional basis for the correlation of DNA mismatch-repair (MMR) deficiency in cancer cells with favorable outcomes. By inactivating MLH1, they showed that these MMR-deficient cells grow in a manner comparable to MMR-proficient cells *in vitro*. However, when transplanted in syngeneic mouse colorectal, breast, and pancreatic mouse cells, there was significantly poorer growth compared with MMR-proficient cancer cells. The group observed increased mutational burden along with persistent renewal of neoantigens that triggered immune surveillance and growth impairment, unlike the profiles seen in MMR-proficient cells. Their interpretation is that DNA-repair processes should be a key target for a novel therapeutic approach, increasing the burden of neoantigens in tumor cells. Tumor debulking could even be coupled with induction of MMR deficiency in order to exploit the neoantigen generation and further enhance immune detection.

Nature 2017; 552:116–120; doi:10.1038/nature.24673

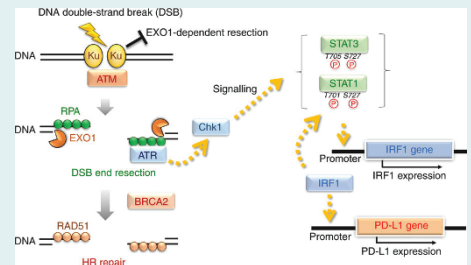


and a glycolytic tumor environment 183 patients with triple-negative breast cancer (TNBC) who underwent surgical resection in the absence of neoadjuvant therapy. The authors found that improved survival was associated with high levels of tumor-infiltrating lymphocytes whereas significantly inferior survival was associated with elevated levels of PD-L1, CD163, and FOXP3. The group used these three markers to differentiate the prognostic significance of tumor-infiltrating lymphocytes. They showed that high FOXP3 and high CD163 in a low-tumor-infiltrating lymphocyte environment denote subgroups of patients with a particularly poor prognosis. An immune-evasive environment and poor prognosis were associated with the expression of MCT4 in the tumor stroma, suggesting a glycolytic tumor environment. These findings suggest that metabolic features of the tumor environment could impact therapeutic sensitivities. Clustering of the markers and the distinction of immune and metabolic markers could identify distinct subtypes of TNBC that have prognostic significance as well as an impact on the effectiveness of immunotherapy.

DNA breakage and PD-L1 expression in cancer cells

Based on evidence indicating that exogenous cellular stress induces PD-L1 upregulation in cancer, Sato *et al* investigated the connection between PD-L1 expression and double-strand breaks (DSBs) in DNA—a critical type of genotoxic stress. This upregulation of PD-L1 requires the activity of several DNA-damaging agents and upregulation of ATM/ATR/Chk1 after DSBs through the repair in living cells. BRCA2 is known to promote homologous recombination in DSB repair, making it a target as a factor that enhances PD-L1 upregulation after DSBs. PD-L1 expression was suppressed by Chk1 inhibition, indicating that PD-L1 in BRCA2-depleted cells requires Chk1 activity. Analysis of PD-L1 expression in neoplastic samples from The Cancer Genome Atlas showed that tumors with mutations in Chk1 had lower PD-L1 expression, although statistical significance was not reached. The group propose that their data suggest prognostic markers that could be further explored in PD-L1-blockade cancer immunotherapy.

Nature Communications 2017; 8:1751; doi:10.1038/s41467-017-01883-9



Evolutionary dynamics in prostate cancer therapy

Zhang *et al* used evolutionary dynamics and game-theory computer simulations to provide a competing methodology for the treatment of metastatic castrate-resistant prostate cancer. As measured via radiographic progression, treatment resistance to standard therapies takes a median of 16.5 months. Using their evolutionary game-theory model in a pilot clinical trial of 11 patients, the group found that they could increase time to progression to 27 months, using 47% of standard dosing. They ran computer simulations of prostate-specific antigen (PSA) under four treatment conditions: no treatment, maximum tolerated dose, metronomic therapy, and their proposed protocol of allowing PSA to fall to 50% of baseline and return before treatment is repeated. Their adaptive model, using patient-specific tumor dynamics to inform on/off treatment cycles, suppresses proliferation of androgen-independent cells and lowers cumulative drug dose. Despite low numbers, the group saw clinical significance in their trial and propose further research in an expanded cohort.

Nature Communications 2017; 8:1816; doi:10.1038/s41467-017-01968-5
Emma Judson contributed to these reviews.

